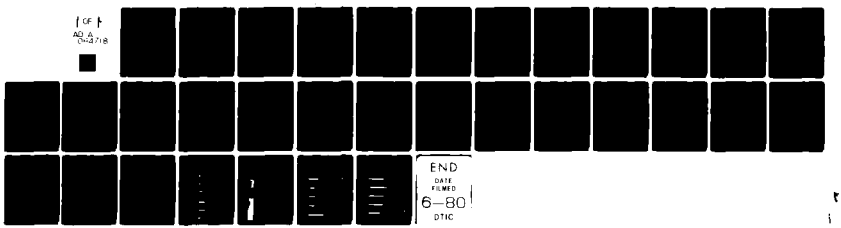
AD-A084 718 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/5
FATTY ACID METABOLISM AND KETOGENESIS IN THE RAT EXPOSED TO STR--ETC(U)
APR 80 J G PACE, F A BEALL, H A NEUFELD

UNCLASSIFIED NL



UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO. AD-A084718

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

Fatty acid metabolism and ketogenesis in the rat exposed to Streptococcus pneumoniae

5. TYPE OF REPORT & PERIOD COVERED

Interim Rept.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

Judith G. Pace, Francis A. Beall, Harold A. Neufeld, Robert W. Wannemacher, Jr.

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS

U.S. Army Medical Research Institute of Infectious Diseases, SGRD-UIP-S
Fort Detrick, Frederick, MD 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

62776A
3M162776A841 00 057

11. CONTROLLING OFFICE NAME AND ADDRESS

U.S. Army Medical Research and Development Command, Office of the Surgeon General
Department of the Army, Washington, DC

12. REPORT DATE

30 April 80

13. NUMBER OF PAGES

25 pages + 5 figures

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)

Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release - distribution unlimited

MAY 27 1980

A

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be published in Canadian Journal of Biochemistry.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Fatty acid metabolism, ketogenesis, carnitine, coenzymeA

80 5 22 018

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Under the dual stress of infectious illness and starvation, the septic host's reduced ability to convert fatty acids to ketone bodies may be partly responsible for the protein-wasting state that accompanies the illness. Previous studies have shown that livers from rats infected with Streptococcus pneumoniae have a decreased ketogenic capacity compared to fasted controls. This study examines possible control sites of hepatic ketogenesis, including hepatic concentrations of coenzyme A, carnitine and malonyl-coenzyme A.. These studies show that during

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

405039

AD A084718

FILE COPY

pneumococcal sepsis, the decreased ketogenic capacity of the liver is accompanied by increased hepatic carnitine concentrations, especially acetylcarnitine, and decreased hepatic coenzyme A (CoA). Infection had no effect on muscle carnitine concentrations or hepatic malonyl-CoA content. These data demonstrate that the decreased rate of ketone body production during infection is not due to a carnitine deficiency. In contrast to malonyl-CoA's regulatory role in the fed and fasted state, the concentration of malonyl-CoA does not appear to be of major importance in determining the rate of ketogenesis during infection. The liver of the fasted-infected rat appears to be shuttling acetyl groups to carnitine, and fatty acyl-CoA away from oxidative pathways toward esterification.

Fatty acid metabolism and ketogenesis in the rat exposed to Streptococcus pneumoniae[1,2]

JUDITH G. PACE,[3] FRANCIS A. BEALL, HAROLD A. NEUFELD, AND ROBERT W. WANNEMACHER, JR.

United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, MD 21701

[1]Preliminary reports of this study were presented at the 63rd Annual Meeting of the American Society of Biological Chemists, Dallas, TX, 1-10 April 1978 and the American Federation of Clinical Research, Newton, MA, 20-21 October 1978.

Excerpts have been taken from a dissertation to be submitted by Judith G. Pace to George Washington University Graduate School, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

[3]To whom requests for reprints should be addressed.

Fatty acid metabolism and ketogenesis in the rat exposed to *Streptococcus pneumoniae*[1,2]

JUDITH G. PACE,[3] FRANCIS A. BEALL, HAROLD A. NEUFELD, AND ROBERT W. WANNEMACHER, JR.

United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, MD 21701

[1]Preliminary reports of this study were presented at the 63rd Annual Meeting of the American Society of Biological Chemists, Dallas, TX, 1-10 April 1978 and the American Federation of Clinical Research, Newton, MA, 20-21 October 1978.

Excerpts have been taken from a dissertation to be submitted by Judith G. Pace to George Washington University Graduate School, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

[3]To whom requests for reprints should be addressed.

ABBREVIATIONS: CoA, coenzyme A; CFU, colony-forming units; s.c., subcutaneous; NAD, nicotinamide-adenine dinucleotide; NADH, nicotinamide-adenine dinucleotide, reduced; BW, body weight.

Pace, J. G., Beall, F. A., Neufeld, H. A. & Wannemacher, Jr., R.W. Fatty acid metabolism and ketogenesis in the rat exposed to Streptococcus pneumoniae

Under the dual stress of infectious illness and starvation, the septic host's reduced ability to convert fatty acids to ketone bodies may be partly responsible for the protein-wasting state that accompanies the illness. Previous studies have shown that livers from rats infected with Streptococcus pneumoniae have a decreased ketogenic capacity compared to fasted controls. This study examines possible control sites of hepatic ketogenesis, including hepatic concentrations of coenzyme A, carnitine and malonyl-coenzyme A. These studies show that during pneumococcal sepsis, the decreased ketogenic capacity of the liver is accompanied by increased hepatic carnitine concentrations, especially acetylcarnitine, and decreased hepatic coenzyme A (CoA). Infection had no effect on muscle carnitine concentrations or hepatic malonyl-CoA content. These data demonstrate that the decreased rate of ketone body production during infection is not due to a carnitine deficiency. In contrast to malonyl-CoA's regulatory role in the fed and fasted state, the concentration of malonyl-CoA does not appear to be of major importance in determining the rate of ketogenesis during infection. The liver of the fasted-infected rat appears to be shuttling acetyl groups to carnitine and fatty acyl-CoA away from oxidative pathways toward esterification.

Introduction

The production of ketone bodies (β-hydroxybutyrate and acetoacetate) is a characteristic metabolic adaptation to starvation in man and experimental animals (1). Ketones can be utilized as energy substrates by tissues such as skeletal muscle and brain. Thus, ketone bodies decrease the glucose requirement of the starved host and spare body protein by reducing the usage of amino acids for glucose synthesis. Neufeld et al. (2) and Blackburn et al. (3) observed a general failure of ketonemic adaptation during severe bacterial and viral infections in man and experimental animals, despite caloric deprivation. This inability of the infected host to utilize fat stores probably contributes to the protein-wasting that accompanies infectious disease.

Ketogenesis is dependent on both the availability of free fatty acids and an alteration in hepatic fat metabolism (4). These two requirements were examined during infection. Fatty acid availability is influenced by the hormonal status of the animal (3-8). However, despite an elevation in plasma insulin concentration during infection, no differences were found in the rate of lipolysis or release and uptake of circulating fatty acids (5-7). Previous studies have shown that livers from fasted-infected rats were less efficient at producing ketone bodies when perfused with a long-chain fatty acid compared to livers from fasted-control rats (5-7). However, no difference was noted when livers from infected rats were perfused with a medium-chain length fatty acid (5-7). These studies suggested that the hepatic mechanisms responsible for the diminished ketogenesis during infection are important from both a biochemical and a physiologic point of view.

Alterations in hepatic ketone production involve a redistribution of fatty acids between esterification and oxidative pathways. Possible control sites of hepatic ketogenesis from long-chain fatty acids include the activation of fatty acids, their carnitine-dependent transport across the mitochondrial membrane (8-10), hepatic carnitine concentrations (10), and cellular concentrations of coenzyme A (CoA) and its derivatives (11-13). Recent studies by McGarry et al. (14) describe malonyl-CoA concentration as another possible regulatory control in hepatic fatty acid oxidation and ketogenesis.

In this study carnitine and CoA were measured in plasma, liver and, skeletal muscle of meal-fed, fasted and fasted-infected rats and in isolated perfused livers. Knowledge concerning the concentrations of these metabolites under these three physiological conditions should help in defining the mechanisms involved in the regulation of fatty acid metabolism and ketogenesis during infection.

Materials and Methods

Animals

Male Fisher-Dunning rats (F-344/Mai f, obtained from Microbiological Associates, Walkersville, MD) weighing 175-200 g, were used in all of the experiments.

In vivo Studies

Between 0800 and 1000 hours rats were meal-fed a standard diet (Teklad Test Diets, Division of ARS/Sprague-Dawley, Madison, WI), which consisted of 10% fat, 26% protein, and 60% carbohydrate. These rats were maintained in a light- and temperature-controlled room [12 h light (1000 to 2200 hours) and 12 h dark and 23 $\pm$ 1°C] during the experiment. After 2 weeks on this feeding schedule, 10 rats were killed at 1000 a.m.; the remainder were inoculated with either 10^4 colony-forming units (CFU) of live or heat-killed Streptococcus pneumoniae type Ia 5 by subcutaneous (s.c.) injection in the nape of the neck. Food was withheld from both groups of rats, with those receiving the heat-killed organisms serving as fasted controls. All fasting rats were kept in wire-bottom cages and allowed water ad libitum. Details concerning preparation of the inoculum and the clinical manifestations of the S. pneumoniae infection have been published elsewhere (15, 16). The progress of the infection was monitored by changes in rectal temperature (Yellow Springs telethermometer).

Ten rats from each group were killed by cervical dislocation 24 h and 48 h postinoculation. The liver was removed and crushed between liquid nitrogen cooled aluminum blocks within 10 s. Blood which had accumulated in the chest cavity after severing the vena cava was collected in heparinized tubes. In one experiment skeletal muscle was removed from the hind leg and frozen within 30 s.

In vitro Studies

Rats were maintained on a commercial diet (Wayne Lab Blox, Allied Inc., Chicago, IL) and housed in rooms maintained at 23 ± 1°C. Livers surgically isolated *in situ* from fed rats and rats exposed to either live or heat-killed *S. pneumoniae* and fasted for 48 h were perfused as described previously by Zenser *et al.* (17). The perfusion medium consisted of 70 mL Krebs-Ringer bicarbonate buffer (pH 7.4), 30 mL washed sheep erythrocytes, 3 g fatty acid-poor bovine albumin (Calbiochem) and 500 units of heparin. The atmosphere in the closed perfusion system was 95% O_2 and 5% CO_2. Perfusion was maintained at approximately 1 mL/min/g; perfusate was recycled. At the end of a 60-min equilibration period, 2.5 mL of a 20 mM solution of either oleic or octanoic acid-albumin complex (7) was injected into the perfusion media. Following the intitial pulse dose, the same fatty acid complex was infused at the rate of 6 mL/h. A 3-ml sample of perfusate was removed at 0, 15, 30, 45, and 60 min and analyzed for free fatty acids (18), 3-hydroxybutyrate (19), and acetoacetate (19). The rate of ketogenesis was calculated from the slope of a plot of total ketones in the media *vs.* time and expressed as μmol/min/100 g body weight. Perfusion with Krebs-Ringer buffer plus fatty acid-poor albumin served as a media control. At the end of the 60-min infusion, the liver was removed, crushed between liquid nitrogen cooled aluminum blocks, and analyzed as discussed below.

In another experiment livers from 48-h fasted-infected rats were perfused with noncirculating medium containing 0.76 mM oleic acid. Livers were perfused at 8 mL/min by frequent adjustment of pressure. At 20 min, L-carnitine was infused at a concentration of 1 mM as described

by McGarry et al. (10). All effluent collected at 5-min intervals for 80 min was analyzed for acetoacetate and β-hydroxybutyrate (19).

Analytical Procedures

Free carnitine and short- and long-chain acylcarnitines were measured in plasma and neutralized tissue extracts according to the radioisotopic assay of Pace et al. (20). Acetylcarnitine was measured in liver by the method of Pearson et al. (21).

Hepatic CoA and acetyl-CoA were measured according to Allred and Guy (22). Long-chain acyl-CoA was hydrolyzed with fatty acid synthetase (23); the released CoA was measured by the enzymatic cycling system containing citrate synthase, malate dehydrogenase and phosphotransacetylase (22). Malonyl-CoA was determined according to McGarry et al. (24).

Hepatic β-hydroxybutyrate and acetoacetate were measured as described by Neufeld et al. (2). Cytoplasmic and mitochondrial $[NAD^+]/[NADH]$ ratios were calculated as described by Williamson et al. (25). Malate and α-glycerophosphate were assayed by the method of Michal and Lang (26). Plasma was analyzed for free fatty acids (18), ketone bodies (19) and insulin (27).

Reagents

Fatty acid synthetase was prepared from rat liver following the procedure of Nepokroeff et al. (28).

All chemicals and solvents were reagent grade. Acetyl-CoA, palmityl-CoA, CoA, acetylcarnitine, carnitine acetyltransferase, citrate synthase, palmityl carnitine, phosphotransacetylase, β-hydroxybutyrate dehydrogenase and acetyl-phosphate were purchased from Sigma. Oleic acid, octanoic acid, carnitine, lactate dehydrogenase and

malate dehydrogenase were purchased from Calbiochem. [1-^{14}C]Acetyl-CoA (specific activity 52 mCi/mmol) was purchased from New England Nuclear.

Statistics

Data were analyzed by one-way analysis of variance. A (p) value of < 0.01 was considered significant under the null hypothesis.

Results

Infection

Rats that received 10^4 CFU of live S. pneumoniae had elevated rectal temperatures at 24 h; by 48 h these body temperatures began to decline. Twenty percent of the rats died between 46 and 48 h, the remainder by 52 h. Plasma ketone body and free fatty acid concentrations were low in infected rats compared to controls, while portal insulin increased above control values (Fig. 1). These changes are characteristic of the pneumococcal infection in rats and are documented elsewhere (2, 15).

In vitro Studies

When oleic acid was added to the recirculating perfusate, the livers from 48-h fasted rats produced ketones at a faster rate than those from normal fed or 48-h fasted-infected rats (2.18 $\pm$ 0.19 vs. 0.64 $\pm$ 0.10 vs. 1.10 $\pm$ 0.10 µmol/min/100 g BW, respectively). No differences were observed in the rates of ketogenesis from octanoic acid infused under the same conditions.

After 60-min perfusion with oleic acid the livers from fed and 48-h fasted rats were frozen, extracted and analyzed for carnitine, CoA and their long- and short-chain derivatives. The data in Fig. 2 show that the concentration of free carnitine was not altered by the infection. However, the concentration of short-chain acylcarnitines, especially acetylcarnitine, and long-chain acylcarnitine, increased in the livers from 48-h fasted-infected rats compared to 48-h fasted control values. The concentrations of free CoA, acetyl-CoA and long-chain acyl-CoA were decreased in the livers from 48-h fasted-infected rats compared to 48-h fasted controls. When octanoic acid was infused, the only significant increase was in the short-chain acylcarnitine fraction (data not shown).

Liver weight is known to decrease during starvation and increase during a pneumococcal infection, while the DNA content remains constant (29, 30). Therefore, results are also expressed on a total liver basis to correct for this weight change. No change occurred in the carnitine content per liver during the 48-h fast (fed, 1273 ± 74 vs. fasted 1395 ± 96 μmol/liver). However, there was a significant increase in the carnitine content of the livers from fasted-infected rats vs. that of control livers (2599 ± 235 vs. 1399 ± 96 μmol/liver). Total coenzyme A decreased during infection (fed, 2139 ± 222; fasted, 1436 ± 126; fasted-infected 648 ± 147 μmol/liver).

The relationship between the ketogenic capacity of livers perfused with oleic acid and their carnitine content per gram of liver is illustrated in Fig. 3. In the 48-hfasted rat, ketone body production increased as did the hepatic carnitine concentration. However, livers from 48-h fasted-infected rats showed a 50% decrease in their rate of ketone production despite an elevated carnitine concentration. Further studies demonstrated that ketogenesis in fasted-infected rats was not altered by the infusion of L-carnitine simultaneously with oleic acid (data not shown).

In vivo Studies

Plasma and muscle concentrations of free carnitine, short- and long-chain acylcarnitines and total carnitine are shown in Table 1. The concentration of free carnitine in plasma of fasted rats decreased at 24 h and approached fed values by 48 h. The increase in total acylcarnitine (short + long) concentration during the fast was reflected in an increase in total carnitine content. Total carnitine was also increased in plasma of fasted-infected rats but the increase was due to an elevation

of both free and acylcarnitine. No significant difference was observed between the fasted and fasted-infected total plasma carnitine concentrations. While Bressler (31) reported a possible lack of muscle carnitine in the metabolic response of the guinea pig to diphtheria toxin, Border et al. (32) showed normal levels of skeletal muscle carnitine in the fasted-septic dog. The present data show that muscle carnitine was minimally affected by bacterial infection. Total carnitine content per gram muscle (Table 1) was not markedly altered by an S. pneumoniae infection; however, long-chain acylcarnitines were significantly decreased during the infection.

Liver concentrations of carnitine and acylcarnitines per gram of liver are presented in Figure 4. Free carnitine decreased by 24 h and remained low in the liver from fasted rats but increased by 48 h in the liver of the fasted-infected rat. Short- and long-chain acylcarnitines increased in the fasted-infected rat above the control values 48 h after inoculation. Total carnitine (acid-soluble + acid-insoluble) was significantly increased in livers from fasted-infected rats whether expressed on a per gram of liver (Fig. 4) or total liver basis (fed, 1736 $\pm$ 138; fasted, 1552 $\pm$ 57; infected, 3747 $\pm$ 172 µmol/liver).

Long-chain acyl-CoA increased in the fasted group compared to fed while both acetyl-CoA and long-chain acyl-CoA decreased in livers from infected rats compared to fasted control rats (Fig. 5). When expressed on a total liver basis there was a significant decrease in the total CoA concentration by 48 h in livers from infected rats (fed, 1291 $\pm$ 124; fasted, 1325 $\pm$ 86; infected, 930 $\pm$ 57 µmol/liver).

Hepatic lactate and pyruvate concentrations were significantly increased in infected rats compared to fasted-controls (Table 2). The cytoplasmic $[NAD^+]/[NADH]$ ratio calculated from these results was not significantly altered by infection. However, there was a significant

increase in the mitochondrial $[NAD^+]/[NADH]$ ratio during an infection compared to fasted-controls. This has been shown to be reflecting changes in both hepatic β-hydroxybutyrate and acetoacetate concentrations (2). The concentration of α-glycerophosphate was slightly decreased in liver from both fasted and fasted-infected rats while malate was significantly increased in the liver from the infected rat.

Discussion

The present studies were undertaken to help understand the hepatic regulatory site(s) responsible for the diminished ketogenesis observed during infection. Although in theory, fatty acid availability could limit ketogenesis, previous perfusion studies showed that despite provision of exogenous fatty acid, livers from fasted-infected rats were less efficient in oxidizing long-chain fatty acids to ketone bodies than were livers from fasted-control rats, while both groups oxidized medium-chain fatty acids at equal rates (5-7). These studies suggested that regulatory sites, such as those proposed by McGarry et al. (10, 14) may be responsible for the decreased ketone production.

McGarry has suggested the use of a regulatory model in which ketogenesis may be influenced by the insulin to glucagon ratio (33), a reduced liver glycogen content and an increased hepatic carnitine concentration. In the fasted-infected rat, plasma insulin and glucagon were both increased with a resulting decrease in the insulin to glucagon ratio (16, 27). While insulin has not been shown to influence the ketogenic capacity of perfused liver from fasted rats, it may play some indirect role in the regulation of ketone production in the infected host (16).

McGarry has proposed that during fasting, compared to the fed condition, hepatic malonyl-CoA and fatty acid synthesis are decreased

subsequent to a decreased plasma insulin to glucagon ratio. The key enzyme involved in this control system is carnitine palmityltransferase I (34-37) which is inhibited by hepatic malonyl-CoA (14). Carnitine acyltransferase activity and hepatic carnitine content increase, resulting in an increased capacity for long-chain fatty acid oxidation and ketogenesis. Certain aspects of the proposed regulatory model of McGarry & Foster (33) were studied in meal-fed, fasted and fasted-infected rats in both *in vivo* and *in vitro* systems.

Preliminary studies on homogenates and mitochondria prepared from livers of fasted-infected rats showed that both preparations were capable of oxidizing long-chain fatty acyl-CoA and fatty acylcarnitine at rates equal to those prepared from fasted-control livers (6, 7). These studies, which indirectly measured the transferase enzymes, suggested that neither transferase I nor II was directly affected by fasting plus infection. However, these studies did not rule out the possibility that cytoplasmic control factors or cellular integrity could be important determinants in the decreased ketone production observed during infection.

Control factors such as malonyl-CoA, carnitine, and coenzyme A have been measured in livers from fasted-infected rats. As was suggested by McGarry *et al.* (14), hepatic malonyl-CoA concentration was decreased in fasted rats compared to fed controls (4.80 ± 0.89 and 13.54 ± 3.10 nmol/g, respectively) (6, 38). However, despite a reduced plasma insulin to glucagon ratio (16) and a decreased malonyl-CoA content (3.25 ± 0.65 nmol/g) in fasted-infected rats compared to fed rats there was a reduced rate of ketogenesis during infection compared to fasting. Thus, malonyl-CoA concentration does not appear to be of major

importance in determining the rate of fatty acid oxidation and ketogenesis during infection, while the fed and fasted conditions are in agreement with those of McGarry et al. (14) and malonyl-CoA could be regulating carnitine palmityltransferase I.

While the carnitine content per gram of liver was increased in fasted compared to fed rats, no significant difference was observed in the carnitine content of fed liver vs. fasted liver when expressed on a total liver basis. This observation is consistent with the results of Brass & Hoppel (39). However, both in vivo and in vitro studies showed increased hepatic carnitine concentration during infection despite a reduced rate of ketogenesis. Thus, while hepatic carnitine concentration could regulate the rate of ketogenesis in fed and fasted rats, it apparently does not play a regulatory role for decreased ketogenesis in liver from fasted-infected rats. The increased hepatic carnitine content during infection may arise from (i) an increased synthesis of carnitine as a consequence of an elevated flux of precursors (lysine and methionine) into the liver (15), or (ii) an increased uptake of carnitine by the liver. Slight decreases in the muscle carnitine pool size could account for the increased liver carnitine concentration. Plasma concentrations of carnitine reflect an increase in acyl derivatives by 24 h and an increase in free carnitine by 48 h postinoculation. Actual prelabeled carnitine flux studies and biosynthesis studies have not yet been performed.

The greatest increase observed during infection was in hepatic short-chain acylcarnitines, especially acetylcarnitine. This increase is consistent with carnitine's capacity to serve as a buffer system for acetyl groups (40, 41) produced either by the mitochondria or the peroxisomes (42) during infection. In either case the subcellular distribution of this derivative would be of importance.

The increase in long-chain acylcarnitine content of liver during perfusion of oleic acid and *in vivo* data suggest that the ability to form long-chain acyl-CoA and acylcarnitine is not affected by infection (transferase enzymes system). These results are in agreement with the mitochondrial studies previously mentioned (6, 7). The actual enzymic activity of carnitine palmityltransferase has not yet been determined, but preliminary studies show no difference in the rate of formation of acyl-CoA from acylcarnitine and CoA (J.G.P.).

Coenzyme A concentration decreased in livers from fasted-infected rats. Under normal conditions changes in acyl- and acetyl-CoA concentrations parallel changes in acylcarnitine and acetylcarnitine content (43). However, during a bacterial infection this was not the case. Long-chain acylcarnitine increased apparently at the expense of acyl-CoA and the acetyl-CoA formed during β-oxidation appeared to be transferred at an increased rate to carnitine, forming acetylcarnitine. The acetylcarnitine can be transported from the mitochondrial matrix or peroxisomes to the cytosol, but it cannot be used in other synthetic pathways unless it reenters the mitochondrial matrix for removal of the carnitine moiety (personal communication, L. L. Bieber). Thus, lower rates of acetyl-CoA oxidation might explain the lower concentration of ketone bodies and CoA observed during the infection.

Accelerated incorporation of fatty acyl-CoA into triglycerides (7) as well as accumulation of long-chain acylcarnitines could account in part for the decrease in long-chain acyl-CoA observed in the perfused and nonperfused livers from infected rats. Since acetyl-CoA was the specific short-chain acyl-CoA measured, the possibility that other acid-soluble derivatives were increased in liver from infected rats

cannot be excluded. Inflammatory diseases have been shown to be accompanied by disturbances in sulfhydryl metabolism (44); such perturbations could contribute to the decrease in hepatic CoA observed by us. Whether the decreased hepatic coenzyme A concentration is reflecting a decreased synthesis, an increased release from hepatocytes or an increased production of other short-chain CoA derivatives, only future studies will determine.

Exton et al. (45) described the antiketogenic effect of lactate in fasted rats and showed that this effect was mimicked by pyruvate. An increase in both lactate and pyruvate concentrations in the fasted-infected rat liver could be reflecting a decreased oxidation of fatty acids and an increased esterificaton to triglyceride and phospholipids. These observations are consistent with previously reported increased incorporation of long-chain fatty acids into liver lipids during perfusion of livers from infected rats with oleic acid (7) and elevated plasma triglycerides (46-47) during bacterial infections.

Fatty acid metabolism during infection is a complex problem. Although the data on fed and fasted rats are in agreement with previous studies of McGarry (10, 14, 24), the fasted-infected rat does not appear to conform to his model for the regulation of hepatic ketogenesis. The present data suggest that the decreased utilization of long-chain fatty acids for ketone body production is not due to a carnitine deficiency. Hepatic carnitine was increased in both the perfused and intact livers from fasted rats infected with S. pneumoniae. Preliminary data (Pace, unpublished) show similar increases in rats exposed to Francisella tularensis. In addition, carnitine infusion, both in vivo (unpublished data) and in vitro, did not increase ketone body production from oleic acid in the infected rat. In view of the current interest in

nutritional supplementation during infectious illness, these data suggest that carnitine supplementation would have no protein sparing effect during infection.

Localization of key intermediates, such as carnitine and CoA between the mitochondrial matrix and the extramitochondrial compartment of the cell could be responsible for the regulation of the rate of ketogenesis in liver from fasted-infected rats. Thus movement of acetyl groups out of the mitochondria coupled with an increased utilization of fatty acyl-CoA for triglyceride synthesis could reduce the amount of acetyl-CoA available for ketone production. Since it is impossible to determine changes in the concentration of these cofactors in an individual compartment of the cell from the present whole tissue measurements future studies will investigate the effect of infection on the subcellular distribution of various derivatives of hepatic carnitine and CoA.

References

1. Cahill, G. F., Jr., Aoki, T. T. & Marliss, E. B. (1971) in Handbook of Physiology, Section 7: Endocrinology, vol. 1, pp. 563-577, American Physiological Society, Washington

2. Neufeld, H. A., Pace, J. A. & White, F. E. (1976) Metabolism 25, 877-884

3. Blackburn, G. L., Flatt, J. P., Clowes, G. H. A., Jr. & O'Donnell, T. E. (1973) Am. J. Surg. 125, 447-454

4. Mayes, P. A. & Felts, J. M. (1967) Nature (London) 215, 716-718

5. Pace J. G., Beall, F. A., Foulke, M. D., Neufeld, H. A. & Wannemacher, R. W., Jr. (1978) Clin. Res. 26, 627A

6. Pace, J. A., Beall, F. A., Neufeld, H. A. & Wannemacher, R. W., Jr. (1977) Fed. Proc. 36, 788

7. Wannemacher, R. W., Jr., Pace, J. G., Beall, F. A., Dinterman, R. E., Petrella, V. J. & Neufeld, H. A. (1979) J. Clin. Invest. 64, 1565-1572

8. Bøhmer, T. (1967) Biochim. Biophys. Acta 144, 259-270

9. Fritz, I. B. & Yue, K. T. N. (1963) J. Lipid Res. 4, 279-288

10. McGarry, J. D., Robles-Valdes, C. & Foster, D. W. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4385-4388

11. Fritz, I. B. (1955) Acta Physiol. Scand. 34, 367-385

12. Wieland, O. & Weiss, L. (1963) Biochem. Biophys. Res. Commun. 13, 26-31

13. Tubbs, P. K., Pearson, D. J. & Case, J. F. A. (1964) Recent Research on Carnitine. Its Relation to Lipid Metabolism, The MIT Press, Cambridge, MA

14. McGarry, J. D., Mannaerts, C. P. & Foster, D. W. (1977) J. Clin. Invest. 60, 265-270

15. Wannemacher, R. W., Jr., Powanda, M. C., Pekarek, R. S. & Beisel, W. R. (1971) Infect. Immun. 4, 556-562

16. Kaminski, M. V., Jr., Neufeld, H. A. & Pace, J. G. (1979) Inflammation 3, 289-294

17. Zenser, T. V., DeRubertis, F. R. & Curnow, R. T. (1974) Endocrinology 94, 1404-1410

18. Dalton, C. & Kowalski, C. (1967) Clin. Chem. 13, 744-751

19. McGarry, J. D., Guest, M. J. & Foster, D. W. (1970) J. Biol. Chem. 245, 4382-4390

20. Pace, J. A., Wannemacher, R. W., Jr. & Neufeld, H. A. (1978) Clin. Chem. 24, 32-35

21. Pearson, D. J., Tubbs, P. K. & Chase, J. F. A. (1974) in Methods of Enzymatic Analysis, 2nd ed. (Bergmeyer, H. U., ed.), vol. 4, pp. 1758-1771, Verlag Chemie Weinheim, Academic Press, New York

22. Allred, J. B. & Guy, D. G. (1969) Anal. Biochem. 29, 293-299

23. Veloso, D. & Veech, R. L. (1974) Anal. Biochem. 62, 449-460

24. McGarry, J. D., Stark, M. J. & Foster, D. W. (1978) J. Biol. Chem. 253, 8291-8293

25. Williamson, D. H., Lund, P. Krebs, H. A. (1967) Biochem. J. 103, 514-527

26. Michal, G. & Lang, G. (1974) in Methods of Enzymatic Analysis, 2nd ed. (Bergmeyer, H. U., ed.), vol. 3, pp. 1415-1418, Verlag Chemie Weinheim, Academic Press, New York

27. George, D. T., Abeles, F. B., Mapes, C. A., Sobocinski, P. Z., Zenser, T. V. & Powanda, M. C. (1977) Am. J. Physiol. 233, E240-E245

28. Nepokroeff, C. M., Lakshmanan, M. R. & Porter, J. W. (1975) Methods Enzymol. XXXV B, 37-44

29. Herrera, E. & Freinkel, N. (1968) Biochim. Biophys. Acta 170, 244-253

30. Thompson, W. L. & Wannemacher, R. W., Jr. (1973) Biochem. J. 134, 79-87

31. Bressler, R. & Wittels, B. (1965) Biochim. Biophys. Acta 104, 39-45

32. Border, J. R., Burns, G. P., Rumph, G. & Schenk, W. G., Jr. (1970) Surgery 68, 175-179

33. McGarry, J. D. & Foster, D. W. (1976) Am. J. Med. 61, 9-13

34. McGarry, J. D. & Foster, D. W. (1971) J. Biol. Chem. 246, 6247-6253

35. McGarry, J. D. & Foster, D. W. (1974) J. Biol. Chem. 249, 7984-7990

36. Woodside, W. F. & Heimberg, M. (1976) J. Biol. Chem. 251, 13-23

37. Christiansen, R. Z. (1977) Biochim. Biophys. Acta 488, 249-262

38. Pace, J. G., Foulke, M. D., Sokol, S., Beall, F. A., Neufeld, H. A. & Wannemacher, R. W., Jr. (1979) Fed. Proc. 38, 354

39. Brass, E. P. & Hoppel, C. L. (1978) J. Biol. Chem. 253, 2688-2693

40. Pearson, D. J. & Tubbs, P. K. (1967) Biochem. J. 105, 953-963

41. Vahouny, G. V., Rodis, S. L., Koch, E. & D'Amato, P. (1969) Adv. Exp. Med. Biol. 4, 279-293

42. Foulke, M. D., Pace, J. G., Canonico, P. G., Little, J. S. & Wannemacher, R. W., Jr. (1979) Clin. Res 27, 589A

43. Bøhmer, T., Norum, K. R. & Bremer, J. (1966) Biochim. Biophys. Acta 125, 244-251

TABLE 1. Effects of S. pneumoniae on plasma and skeletal muscle carnitine

Variable	Fed	Fasted-Control		Fasted-Infected	
		24 h	48 h	24 h	48 h
Plasma (nmol/mL)					
Free	75 ± 5	45 ± 2[d]	58 ± 4	32 ± 2[d]	93 ± 14[b,d]
Short + long	33 ± 5	57 ± 8	70 ± 8[d]	85 ± 16[a,d]	60 ± 6[c]
Total pool	104 ± 6	102 ± 6	129 ± 9	134 ± 6[a,c]	153 ± 17[d]
Muscle (nmol/g)					
Free	775 ± 57	658 ± 40[c]	800 ± 86	618 ± 22[c]	742 ± 34
Short	523 ± 118	414 ± 51	353 ± 51	411 ± 28	351 ± 98
Long	189 ± 21	197 ± 14	224 ± 16	146 ± 20[a]	168 ± 10[a]
Total pool	1451 ± 106	1131 ± 50[c]	1258 ± 75	1175 ± 29	1249 ± 77

NOTE: Values are means ± standard error of the mean.

[a] $p < 0.05$ compared to fasted-controls.

[b] $p < 0.01$ compared to fasted-controls.

[c] $p < 0.05$ compared to fed controls.

[d] $p < 0.01$ compared to fed controls.

44. Steinetz, B., Giannina, F. & Butler, M. (1973) J. Pharmacol. Exp. Therap. 185, 139-149

45. Exton, J. H., Corbin, J. G. & Harper, S. C. (1972) J. Biol. Chem. 247, 4996-5003

46. Kaufmann, R. L., Matson, C. F., Rowberg, A. H. & Beisel, W. R. (1976) Metabolism 25, 615-624

47. Canonico, P. G., Ayala, E., Rill, W. & Little, J. S. (1977) Am. J. Clin. Nutr. 30, 1359-1363

TABLE 2. Effects of S. pneumoniae on hepatic metabolites and the $[NAD^+]/[NADH]$ ratios

Variable	Fed	Fasted-Control		Fasted-Infected	
		24 h	48 h	24 h	48 h
Liver weight (g)	8.82 ± 0.32	8.24 ± 0.26	6.41 ± 0.19	7.79 ± 0.34	8.28 ± 0.23[a]
Lactate (μmol/g)	1.38 ± 0.10	0.40 ± 0.04	0.33 ± 0.06	0.42 ± 0.04	0.67 ± 0.03[a]
Pyruvate (μmol/g)	0.088 ± 0.008	0.031 ± 0.002	0.031 ± 0.003	0.034 ± 0.003	0.058 ± 0.003[a]
β-OH[b] (μmol/g)	0.17 ± 0.01	0.78 ± 0.08	1.54 ± 0.07	0.59 ± 0.07	0.37 ± 0.02[a]
Acetoacetate (μmol/g)	0.09 ± 0.01	0.25 ± 0.03	0.31 ± 0.02	0.25 ± 0.03	0.15 ± 0.01[a]
Malate (μmol/g)	0.45 ± 0.04	0.40 ± 0.07	0.35 ± 0.08	0.34 ± 0.06	0.54 ± 0.02
α-G-PO_4[c] (μmol/g)	0.21 ± 0.04	0.20 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.18 ± 0.02
$[NAD^+]/[NADH]$ Ratio					
Mitochondria	11.23 ± 1.45	6.39 ± 0.52	4.26 ± 0.42	8.91 ± 0.74	8.24 ± 0.45
Cytoplasm	580 ± 55	750 ± 62	965 ± 123	774 ± 65	790 ± 57

NOTE: Values are mean ± standard error of the mean.

[a] $p < 0.01$ vs. Fasted-controls at same time period.

[b] β-Hydroxybutyrate.

[c] α-Glycerophosphate.

Figure Legends

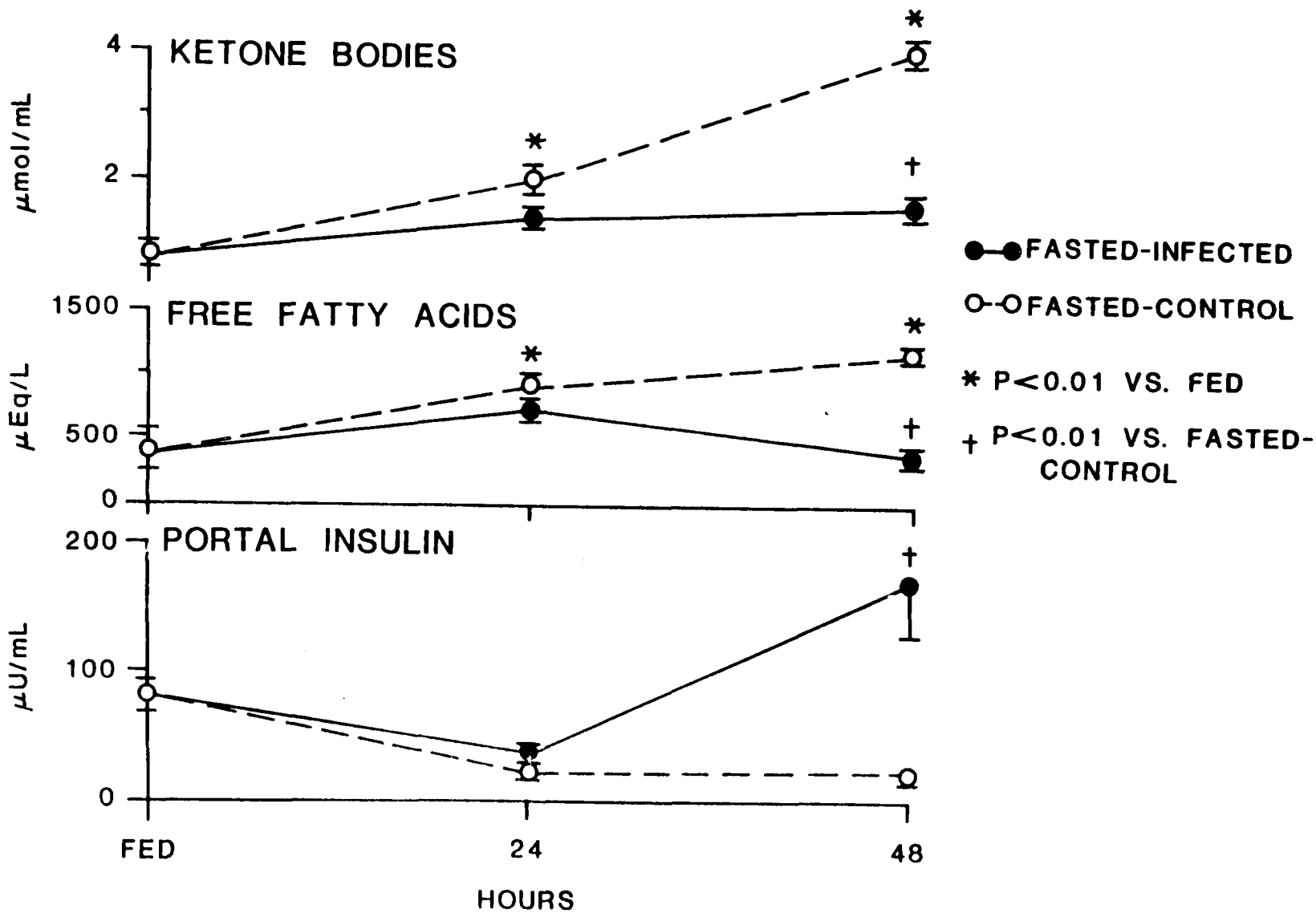
FIG. 1. Effect of S. pneumoniae infection on rat plasma ketone bodies, free fatty acid and portal insulin concentrations. Values are expressed as the mean ± SEM for 10 rats per group.

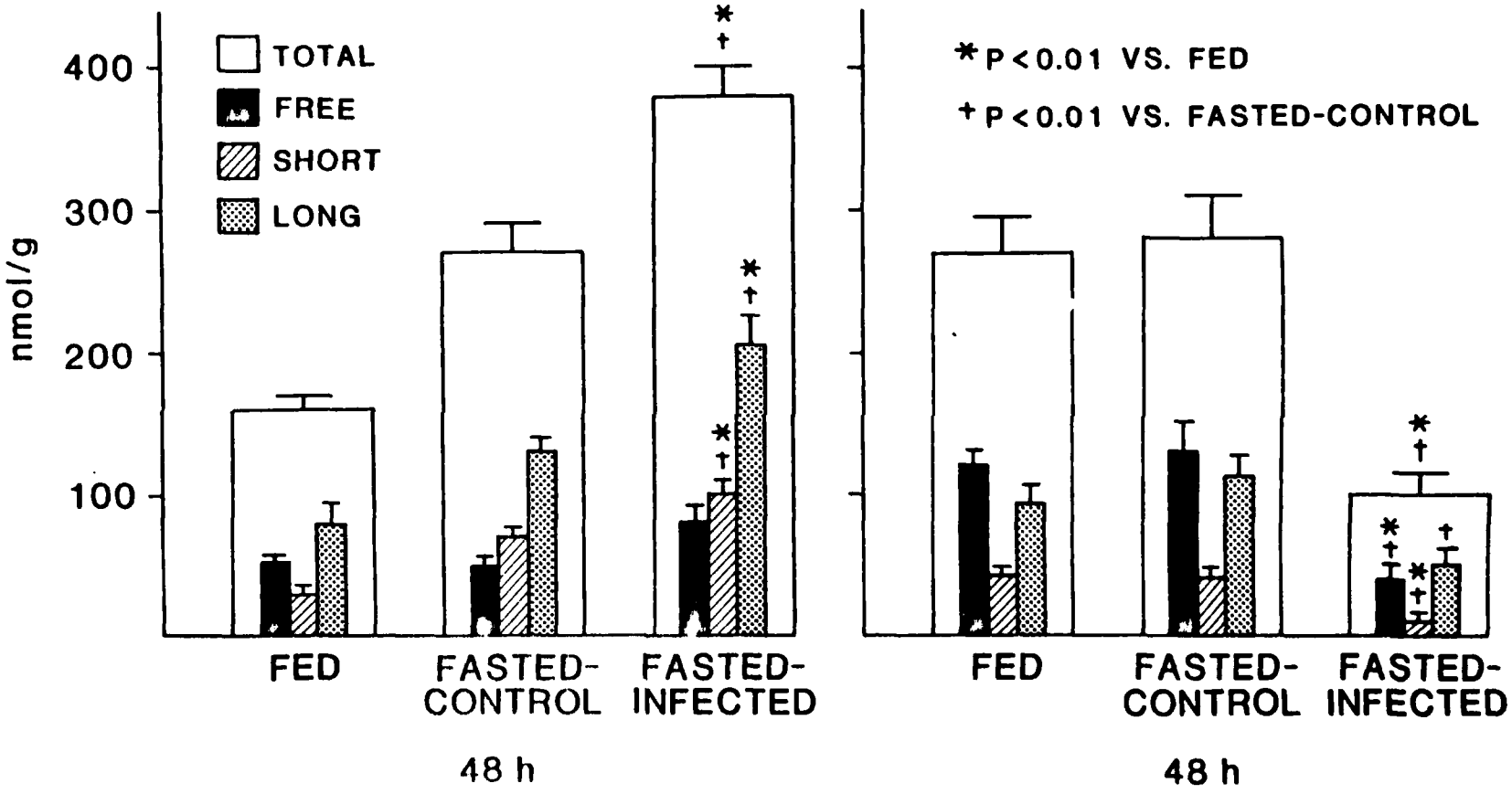
FIG. 2. Carnitine and CoA content of livers from fed, fasted-control rats and fasted rats infected with S. pneumoniae and perfused with oleic acid as described in the Methods section. Values are expressed as the mean ± SEM for from 6 to 10 livers per group.

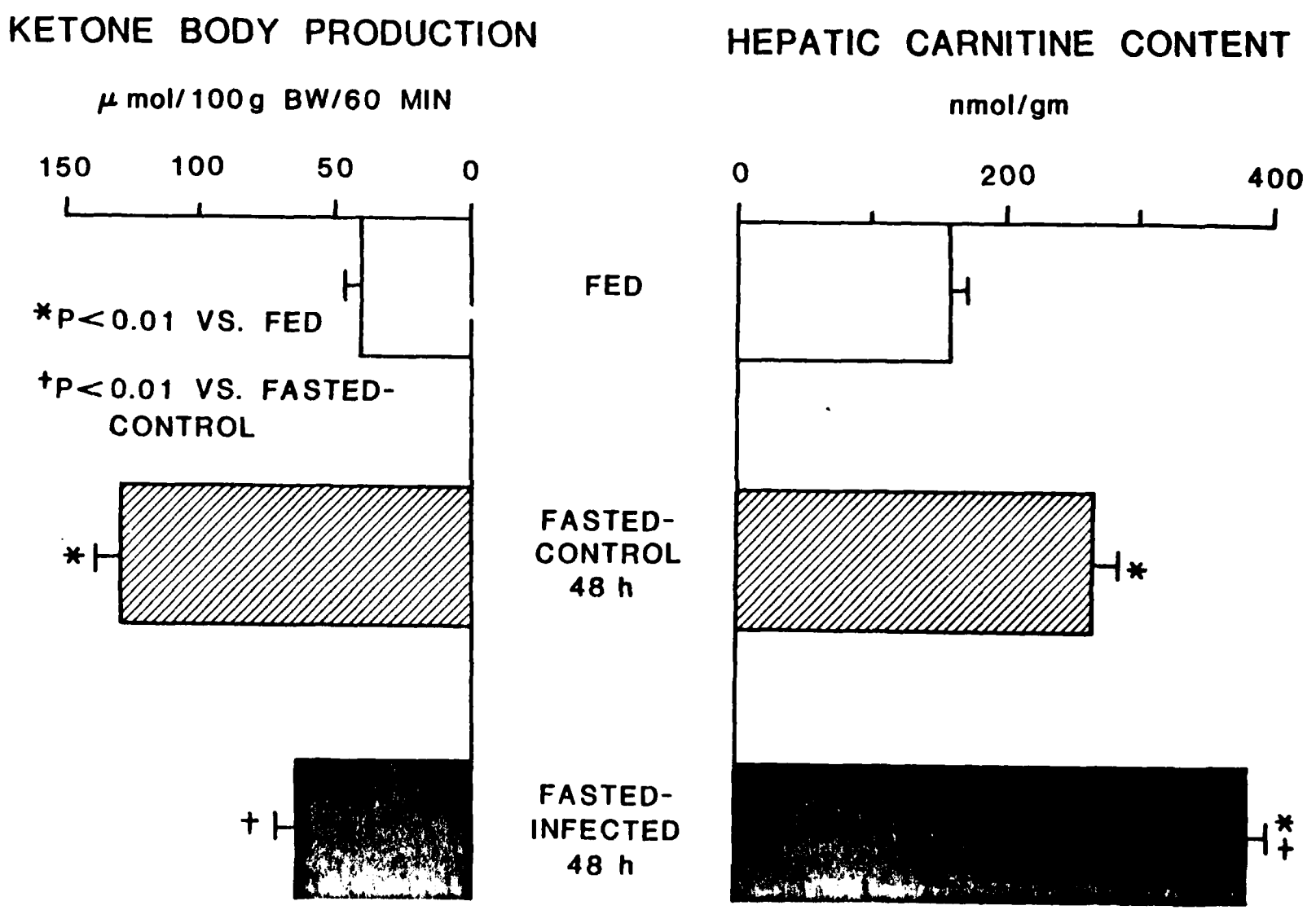
FIG. 3. The relationship between ketone body production and hepatic carnitine content of livers from fed and fasted-control rats and fasted rats infected with S. pneumoniae. The livers were perfused with oleic acid, frozen and assayed as described in text. Each bar represents the mean ± SEM of 6 to 10 experiments.

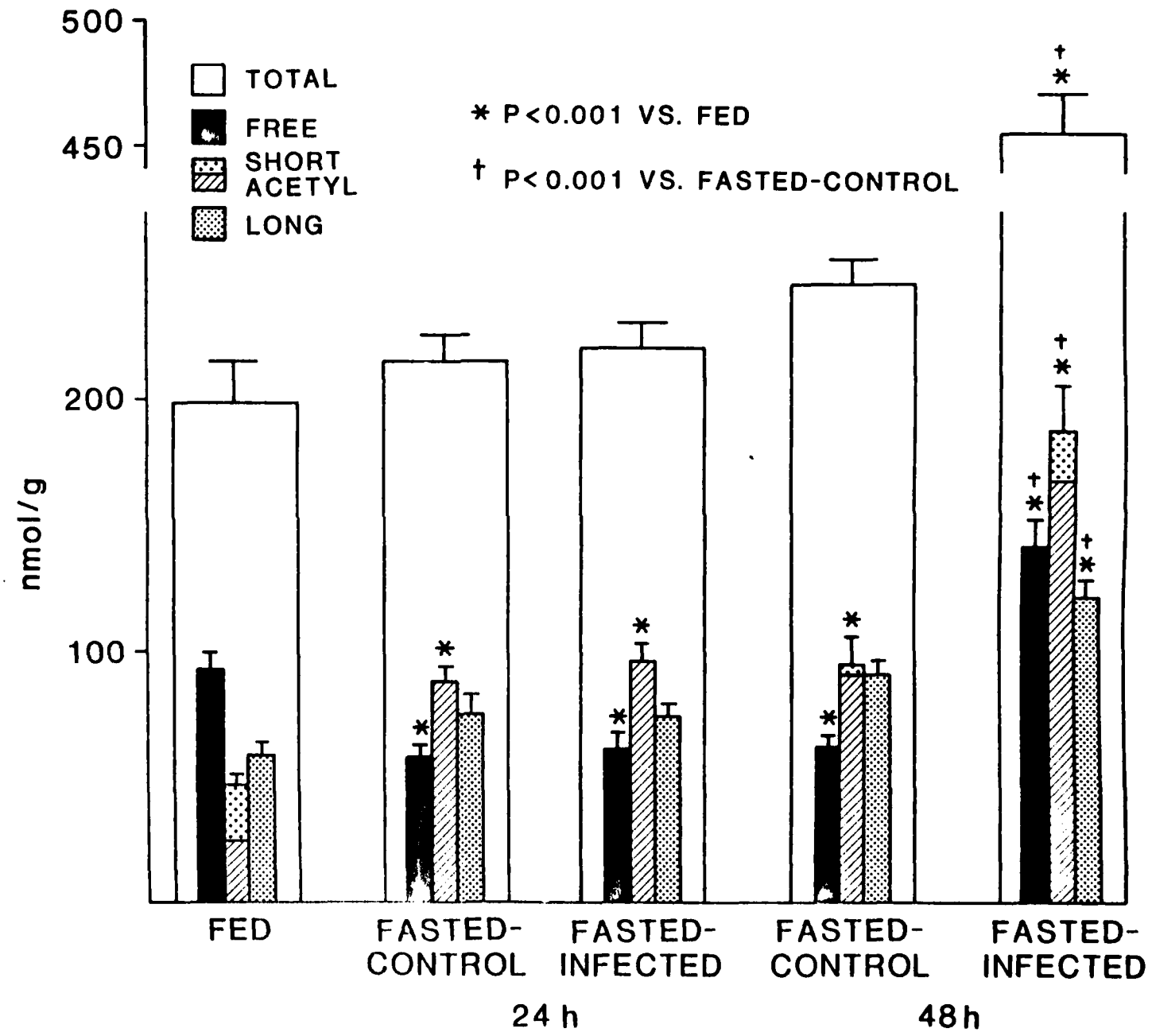
FIG. 4. Concentration of carnitine and its acyl derivatives in livers from fed, fasted-control and fasted-infected rats. Each bar represents the mean ± SEM for 10 rats.

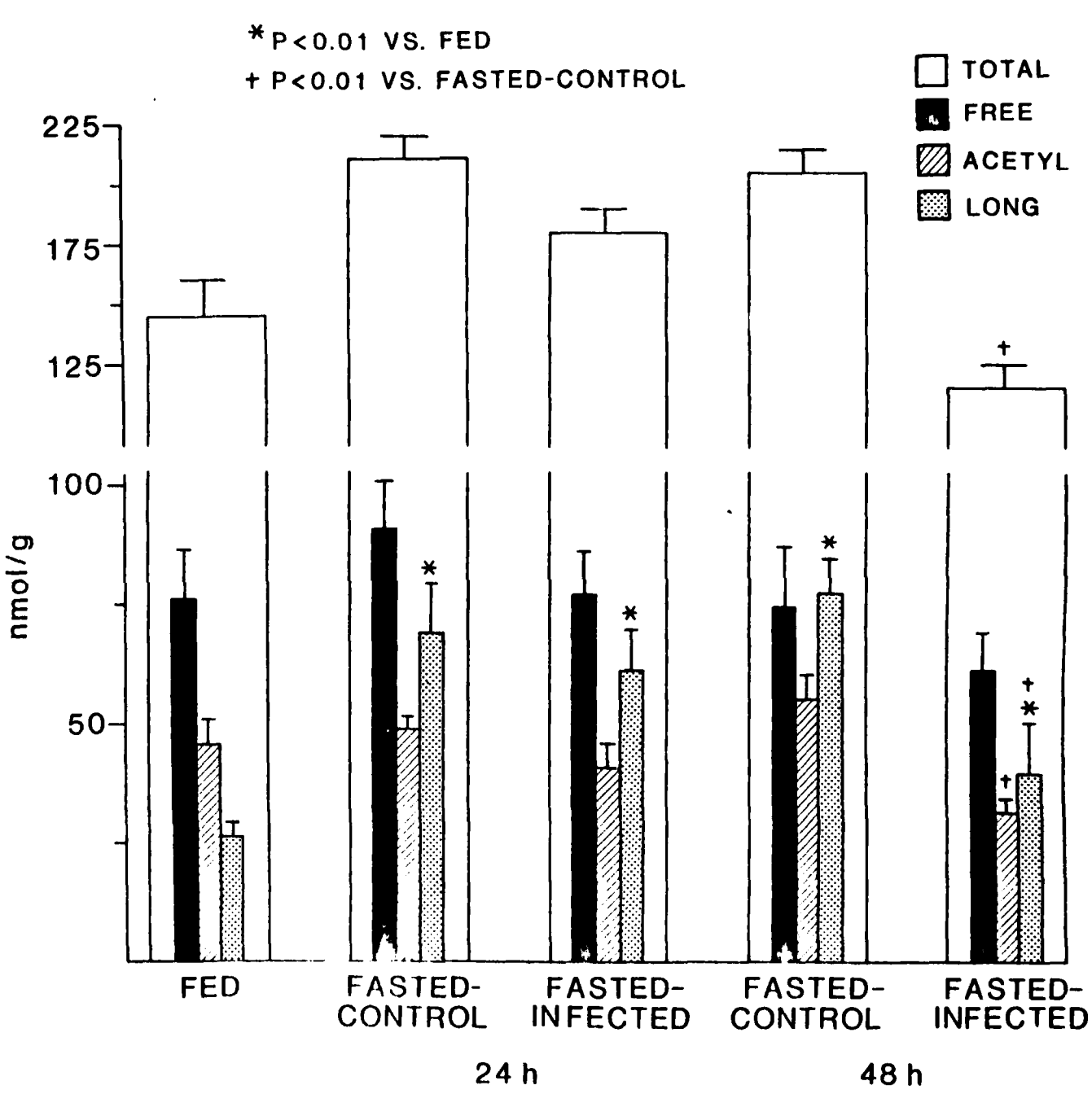
FIG. 5. Concentration of CoA and its acyl derivatives in livers from fed, fasted-control and fasted-infected rats. Each bar represents the mean ± SEM for 10 rats.


KETONE BODIES
4
2
µmol/mL
FREE FATTY ACIDS
1500
500
0
µEq/L
PORTAL INSULIN
200
100
0
µU/mL
FED
24
48
HOURS
FASTED-INFECTED
FASTED-CONTROL
* P<0.01 VS. FED
+ P<0.01 VS. FASTED-CONTROL


TOTAL
FREE
SHORT
LONG
nmol/g
400
300
200
100
* P < 0.01 VS. FED
† P < 0.01 VS. FASTED-CONTROL
FED
FASTED-CONTROL
FASTED-INFECTED
48 h
FED
FASTED-CONTROL
FASTED-INFECTED
48 h


KETONE BODY PRODUCTION
μ mol/100g BW/60 MIN
150
100
50
0
*P<0.01 VS. FED
†P<0.01 VS. FASTED-CONTROL
FED
FASTED-CONTROL 48 h
FASTED-INFECTED 48 h
HEPATIC CARNITINE CONTENT
nmol/gm
0
200
400


500
450
200
100
nmol/g
TOTAL
FREE
SHORT ACETYL
LONG
* P<0.001 VS. FED
† P< 0.001 VS. FASTED-CONTROL
FED
FASTED-CONTROL
FASTED-INFECTED
FASTED-CONTROL
FASTED-INFECTED
24 h
48h


* P<0.01 VS. FED
+ P<0.01 VS. FASTED-CONTROL
TOTAL
FREE
ACETYL
LONG
225
175
125
100
50
nmol/g
FED
FASTED-CONTROL
FASTED-INFECTED
FASTED-CONTROL
FASTED-INFECTED
24 h
48 h